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PHYLOGENETIC RELATIONSHIPS AMONG MEMBERS OF THE *HYBOPSIS AMBLOPS* SPECIES GROUP (TELEOSTEI: CYPRINIDAE)

KATE SHAW, E. O. WILEY, AND TOM A. TITUS[1]

*Division of Ichthyology, Natural History Museum and
Department of Systematics and Ecology, The University of Kansas,
Lawrence, Kansas 66045-2454, USA*

ABSTRACT Phylogenetic relationships among the *Hybopsis amblops* species group were examined using allozymic variation at 23 presumptive gene loci in combination with 14 morphological characters. Qualitative analyses using allozymes and morphological data resulted in up to 14 equally parsimonious trees, the strict consensus of which is (*H. hypsinotus* (*H. rubrifrons* (*H. amblops* (*H. lineapunctata, H. amnis, H. winchelli, H.* sp.)))) for the ingroup taxa. Quantitative analyses incorporating estimates of allelic frequencies revealed a maximum difference of only 1.06 steps among 15 alternative trees, of which (*H. hypsinotus* (*H. rubrifrons* (*H. amblops* (*H.* sp. (*H. amnis* (*H. winchelli, H. lineapunctata*)))))) is the shortest tree. *Hybopsis amnis* and a clade consisting of *H. lineapunctata* and *H. winchelli* are part of a group of species whose vicariance is associated with the Mississippi River Basin. In addition, this analysis indicates a possible vicariance event above and below the Fall Line within the Mobile System between *H. winchelli* and *H. lineapunctata*. *Hybopsis amblops* and *H. amnis* may not be sister taxa, and therefore, may not show a vicariant distribution. A sister-group relationship between *H. winchelli* and *H.* sp., an undescribed species east of the Mobile Drainage, was not supported, providing additional evidence that *H.* sp. is an independent lineage awaiting formal description. Morphological and allozymic data provide complementary phylogenetic information in this study.

Key Words: Hybopsis; Allozymes; Historical biogeography; Phylogeny; Polymorphism.

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[1] Present address: Department of Biology, University of Oregon, Eugene, Oregon 97403, USA.

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The Cyprinidae includes more than 2010 species of minnows and carps found throughout North America, Africa, and Eurasia (Nelson, 1994). In North America, there are 302 described and undescribed species in at least 50 genera (Burr and Mayden, 1992).

Composition of the minnow genus *Hybopsis* has been controversial for most of its taxonomic history (Reno, 1969). The genus *Hybopsis* was erected by Agassiz (1854) for an Alabama cyprinid he named *H. gracilis* (later recognized as *H. amblops*). Jordan and Evermann (1896) expanded *Hybopsis* to include the previously recognized genera *Hybopsis*, *Ceratichthys*, *Nocomis*, *Erinemus*, and *Erimystax*. Their subgenus *Hybopsis* included the species *H. amblops* (including *H. winchelli*), *H. labrosus* (later recognized as *H. labrosa*), *H. hypsinotus*, and *H. storerianus* (later recognized as *Macrhybopsis storeriana*). Jordan (1924; 1929) chose to elevate the subgenera *Hybopsis*, *Nocomis*, *Erinemus*, *Erimystax*, *Extrarius*, *Macrhybopsis*, and *Yuriria* to generic status. Jordan (1929) placed most of the species previously in the subgenus *Hybopsis* (*H. amblops*, *H. labrosus*, *H. rubrifrons*, *H. hypsinotus*, and *H. storerianus*) in his resurrected genus *Erinemus*. Jordan (1929) and Jordan et al. (1930) placed the species *H. gracilis* with the *Alburnops* and the *Chriope* species groups (of *Notropis*) in the newly reorganized genus *Hybopsis*. In 1951, Bailey placed the genera *Nocomis*, *Hybopsis*, *Yuriria*, *Erimystax*, *Platygobio*, *Macrhybopsis*, *Extrarius*, *Oregonichthys*, and *Couesius* in a much-expanded genus *Hybopsis*. This larger version of the genus *Hybopsis*, although controversial at the time, remained mostly intact until Lachner and Jenkins (1967) elevated *Nocomis* once more to generic status. Bailey et al. (1970) listed 16 species in the genus *Hybopsis*, but noted that some authors would place some of these taxa in *Erimystax*, *Extrarius*, *Macrhybopsis*, *Oregonichthys*, and *Platygobio*. These authors also elevated *Couesius* to generic status. Jenkins and Lachner (1971) and Lachner and Jenkins (1971) reviewed the composition and characterization of the genus *Hybopsis*. They recognized seven subgenera: *Hybopsis*, *Yuriria*, *Erimystax*, *Platygobio*, *Macrhybopsis*, *Extrarius*, and *Oregonichthys*. The subgenus *Hybopsis* of Jenkins and Lachner (1971) comprised *H. amblops*, *H. rubrifrons*, *H. hypsinotus*, *H. labrosa*, *H.* sp., cf. *labrosa* (now *H. zanema*), and *H. storeriana*. Clemmer (1971) recognized the genus *Hybopsis* and added *H. winchelli*, *H.* sp., cf. *winchelli*, *H. lineapunctata* (described in Clemmer and Suttkus, 1971), and *H. amnis*. Robins et al. (1980) listed 17 species in the genus *Hybopsis*, including two newly recognized species, *H. lineapunctata* and *H. zanema*. Other differences from the 1970 list included removal of *H. harperi* to *Notropis* and recognition of *H. insignis*.

The composition of the genus *Hybopsis*, indeed even its validity, remains controversial. Mayden (1989) revised the genus *Hybopsis*, removed *H.*

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storeriana to *Macrhybopsis*, and added *H. boucardi*, *H. calientis*, *H. dorsalis*, *H. longirostris*, *H. sabinae*, *H.* sp., cf. *labrosa*, *H. alborus*, and *H. bifrenatus*. He based this revision on three morphological characters shared by this group of 17 species plus *Ericymba buccata*. He also partially resolved relationships among members of the genus *Hybopsis*. In contrast, Robins et al. (1991) and Coburn and Cavender (1992) did not recognize this genus at all. They considered *Notropis amblops*, *N. hypsinotus*, *N. lineapunctata*, *N. rubrifrons*, *N. winchelli*, and *N. amnis* to be members of the genus *Notropis*, subgenus *Hybopsis*. Page and Burr (1991) recognized *H. amblops*, *H. hypsinotus*, *H. lineapunctata*, *H. rubrifrons*, *H. winchelli*, *H. amnis*, *H. labrosa*, and *H. zanema* as the sole members of the genus *Hybopsis*. Wiley and Titus (1992) examined relationships among members of the *H. dorsalis* species group and placed *Ericymba* in synonymy with *Hybopsis*. Mayden et al. (1992) included as many as 25 described and undescribed species in the genus *Hybopsis*.

Regardless of the classification, six species comprise a monophyletic group in recent phylogenetic studies (Mayden, 1989; Coburn and Cavender, 1992). These species and an undescribed species are here placed in the *Hybopsis amblops* species group. They are distributed largely east of the Mississippi River Basin, although one taxon is distributed from the Mississippi River Basin westward along the Gulf Coastal Plain to the Guadalupe River Basin (Lee et al., 1980). These seven species are *H. amblops* (Rafinesque, 1820), *H. hypsinotus* (Cope, 1870), *H. rubrifrons* (Jordan, 1877), *H. lineapunctata* Clemmer and Suttkus, 1971, *H. amnis* (Hubbs and Greene, 1951) (in Hubbs, 1951), *H. winchelli* (Girard, 1857), and an undescribed species first noted by Clemmer (1971), *H.* sp., cf. *winchelli*.

The distributions of *Hybopsis amblops*, *H. amnis*, *H. winchelli*, and *H.* sp. may be the result of vicariant allopatric speciation (Wiley and Mayden, 1985). If *H. amblops* and *H. amnis* are sister taxa, they would join a group of species whose vicariance event is associated with the Mississippi River. *Hybopsis winchelli* and its presumed sister *H.* sp. occur from the eastern tributaries of the Mississippi River to the Ocklocknee River in Florida. These two forms are not found in sympatry; the Mobile Bay Basin forms a boundary between them.

To address the nature of speciation events, taxonomic issues, and character evolution, a phylogeny is needed. However, phylogenetic relationships among the species of the *Hybopsis amblops* species group have not been explicitly investigated. The purpose of this investigation is to further evaluate relationships among species of the *H. amblops* species group using allozymic data in combination with previously described morphological variation.

METHODS AND MATERIALS

Taxa examined.—All seven members of the *Hybopsis amblops* species group are included in the ingroup.

The first taxonomic outgroup is *Hybopsis zanema*, which represents the *H. zanema* species group, composed of *H. zanema, H. labrosa* and an undescribed species, the thinlip chub. The monophyly of this group is supported by four morphological synapomorphies (Mayden, 1989). The monophyly of a group comprising the *H. amblops* species group and the *H. zanema* species group is supported by six morphological synapomorphies (Mayden, 1989) as well as allozyme data (Dimmick, 1993).

Because of poor phylogenetic resolution of more distantly related taxa, choice of a second taxonomic outgroup was unclear. For this reason, the outgroup substitution method of Donoghue and Cantino (1984) was employed. Each of three species, *Hybopsis boucardi, H. dorsalis,* and *H. buccata,* were substituted in turn, as the second taxonomic outgroup in cladogram construction. *Hybopsis boucardi* was chosen to represent the Mexican group of *Hybopsis* (sensu Mayden, 1989; Mayden et al., 1992). Mayden (1989) presented a single morphological character that unites *H. boucardi* with a group consisting of the *H. amblops* and *H. dorsalis* species groups but noted that this synapomorphy is convergent with the genus *Cyprinella*. *Hybopsis dorsalis* was chosen to represent the *H. dorsalis* species group (Wiley and Titus, 1992). *Hybopsis buccata* was designated the outgroup by Wiley and Titus (1992) for their analysis of the phylogeny of the *H. dorsalis* species group, but the relationship of *H. buccata* to the *H. dorsalis* species group and the rest of the genus *Hybopsis* is unclear.

Cyprinella lutrensis was used as the third taxonomic outgroup. This species is clearly outside the genus *Hybopsis*, and these two genera are demonstrably monophyletic and part of a larger group of North American cyprinids that share an open posterior myodome (Mayden, 1989).

Enzyme electrophoresis.—Fishes were collected by seining, frozen immediately in liquid nitrogen, transported to the laboratory, and stored at -70°C for up to 6 yr (see Appendix—Specimens Examined). Skeletal muscle and brain/eye tissues were dissected and homogenized separately in a 1:1 (v:v) mixture of tissue and 0.01 M Tris, 0.001 M EDTA, and 0.001 M mercaptoethanol, pH 6.8. Homogenates were centrifuged at 15,000 ×*g* for 10 min at 5°C. Within 72 h, the supernatant fractions were electrophoresed at 5°C on horizontal starch gels composed of 12% hydrolyzed potato starch (Starch Art Corp.). Histochemical staining protocols did not differ substantially from those of Murphy et al. (1990).

Enzyme nomenclature follows the recommendations of the International Union of Biochemistry Nomenclature Committee (1984) and locus nomenclature follows the recommendations of Buth (1983). Enzymes, loci, tissue sources, and electrophoretic conditions are listed in Table 1. Twenty-seven presumptive gene loci were visualized by histochemical staining. Each electromorph was considered an allele, and for each locus these alleles were coded *a, b, c*, etc., in order of increasing anodal mobility. These designations are relevant to this study only. Twenty-three variable loci were then each considered independent transformation series (TS), and each allele for a locus was considered a unique character (TS 1–TS 23, Table 2).

Morphological characters.—Fourteen morphological characters were included in this study. These characters are redescribed here using the character-state assignments for each taxon given by Mayden (1989). Transformation series and character numbers refer to those presented in Table 2.

TS 24—The premaxillary process of the maxilla is high (1) or low (0). All members of the genus *Hybopsis* (sensu Mayden, 1989) share Character 1, as do some members of *Cyprinella. Cyprinella lutrensis* has Character 0.

TS 25—The maxillary process of the palatine is an expanded, anterolaterally directed flange that is broadly connected to the body of the palatine (1), or if expanded, is an anteriorly directed, fingerlike process, not as broadly connected to the body of the palatine (0). All *Hybopsis* share Character 1, as do members of the *Notropis volucellus* species group.

TS 26—The palatine has a long, deep, anteroventrally-to-posterodorsally directed pocket between the vomerine and supraethmoid processes on the mesial surface. This pocket is posteriorly restricted by a mesially directed ridge from the supraethmoid process (1), or it is a small, shallow, depressed area not posteriorly restricted by a ridge (0). The preethmoid lies in this pocket or depressed area. Members of the genus *Hybopsis* and the *Notropis volucellus* species group share Character 1.

TS 27—The body of the maxilla is high (1) or low, forming a narrow isthmus between the ascending process and the premaxillary and rostral processes (0). Character 1 occurs in *Hybopsis boucardi, H. zanema*, all members of the *H. amblops* species group, and some members of the genus *Cyprinella. Cyprinella lutrensis* has a comparatively low maxillary body (Character 0).

TS 28—The bony portion of the mandibular canal partially or entirely overlies the retroarticular bone (1), or entirely overlies the anguloarticular bone (0). Character 1 is shared by the *Hybopsis zanema* and *H. amblops* species groups. This transformation series also was discussed by Coburn (1982).

TS 29—The interhyal is long (1) or short and stout (0). Character 1 is shared by the *Hybopsis amblops* and *H. zanema* species groups.

Table 1. Enzymes, loci, International Union of Biochemistry Nomenclature Committee numbers, tissue sources, and electrophoretic conditions for examination of the *Hybopsis amblops* species group.

Enzyme	IUBNC No.	Locus	Tissue source	Electrophoretic conditions*
Aconitate hydratase	4.2.1.3	M-Acon-A	Muscle	A
Adenosine deaminase	3.5.4.4	Ada-A	Muscle	A
Adenylate kinase	2.7.4.3	Ak-A	Brain/eye	B
Calcium binding proteins	Nonspecific	Cbp-1	Muscle	C
		Cbp-2	Muscle	C
Creatine kinase	2.7.3.2	Ck-A	Muscle	C
		Ck-B	Brain/eye	B
		Ck-C	Brain/eye	B
Fumarate hydratase	4.2.1.2	Fum-A	Muscle	D
General protein	Nonspecific	Gp-1	Muscle	C
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6pdh-B	Brain/eye	D
Glucose-6-phosphate isomerase	5.3.1.9	Gpi-A	Muscle	A
		Gpi-B	Muscle	A
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	Gapdh-A	Muscle	D
		Gapdh-B	Brain/eye	D
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3pdh-A	Muscle	D
Isocitrate dehydrogenase	1.1.1.42	M-Icdh-A	Muscle	D
L-Lactate dehydrogenase	1.1.1.27	Ldh-A	Brain/eye	B
		Ldh-B	Brain/eye	B
Malate dehydrogenase (NAD$^+$-dependent)	1.1.1.37	M-Mdh-A	Brain/eye	D
		S-Mdh-A	Brain/eye	D
		S-Mdh-B	Muscle	D
Malate dehydrogenase (NADP$^+$-dependent)	1.1.1.40	S-Me-A	Muscle	D
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh-A	Brain/eye	B
Triose phosphate isomerase	5.3.1.1	Tpi-A	Brain/eye	B
		Tpi-B^1	Brain/eye	B
		Tpi-B^2	Brain/eye	B

*A: Tris-citrate I pH 7.0 (a discontinuous buffer system using 0.22 M TRIS and 0.09 M citric acid for the electrode and 0.01 M TRIS and 0.003 M citric acid for the gel), 5.88 V/cm 8 h; B: Tris-citrate I-NADP pH 7.0, 5.88 V/cm 8 h; C: Histidine-citrate pH 7.0 (Fildes and Harris, 1966), 5.59 V/cm 8 h; D: Tris-citrate II pH 8.0 (Selander et al., 1971), 5.59 V/cm 8 h.

TS 30—Hypobranchial 1 is enlarged, much larger than Hypobranchial 2 (1), or small, nearly equal in size to Hypobranchial 2 (0). Character 1 is shared by the *Hybopsis zanema* and *H. amblops* species groups.

TS 31—Basibranchial 1 is elongate, approaching Basibranchial 2 in length (1), or short, about half the length of Basibranchial 2 (0). Character 1 is shared by the *Hybopsis amblops* and *H. zanema* species groups.

TS 32—The posterior portion of the parasphenoid is broad, such that near the foramen for the carotid artery it is much wider than near the opening of the posterior myodome (1), or narrow, such that the posterior external margins are nearly parallel (0). Character 1 is considered unique to the *Hybopsis zanema* and *H. amblops* species groups among all North American cyprinids.

TS 33—In life, the orbit and eye are oval (1) or round (0). Character 1 is shared by the *Hybopsis amblops* and *H. zanema* species groups.

TS 29–TS 33 were originally described by Wiley and Mayden (1985) as diagnostic of the *Hybopsis amblops* species group, but Mayden (1989) showed that they are synapomorphic for a group comprising the *H. amblops* and *H. zanema* species groups.

TS 34—The horizontal plate of the urohyal is truncated, usually broadest posteriorly and longest in the midline (1), or elongate laterally to form two lateral processes and usually broadest from midlength anteriorly (0). Character 1 is shared by the *Hybopsis amblops* species group, but also occurs in some *Notropis* and *Phenacobius*.

TS 35—The urohyal, near the neck, has two pointed, thickened, posterolaterally directed processes (1), or lacks these processes (0). Members of the *Hybopsis amblops* species group share Character 1 (Wiley and Mayden, 1985), but it is also present in *Macrhybopsis storeriana*, some *Phenacobius*, and some *Notropis hudsonius* (Mayden, 1989).

TS 36—The vomerine process of the palatine is posteriorly directed (1), ventrally directed (2), or anteriorly directed (0). *Hybopsis amnis, H. amblops, H. winchelli,* and *H.* sp. share Character 1. Character 2 is unique to *H. lineapunctata.* All other North American cyprinids have Character 0 (Mayden, 1989).

TS 37—The depression on the anterodorsal face of the palatine between the supraethmoid and maxillary processes is well developed (1) or shallow (0). Character 1 occurs in *Hybopsis lineapunctata, H. amnis, H. winchelli,* and *H.* sp.

Phylogenetic analyses.—The underlying methodology for this study is phylogenetic parsimony analysis (Hennig, 1966; Kluge and Farris, 1969; Wiley, 1981; Farris, 1983; Churchill et al., 1985; Farris and Kluge, 1985,

Table 2. Character matrix used in the qualitative PAUP analyses for members of the *Hybopsis amblops* species group. The first taxonomic outgroup was *H. zanema* and the second taxonomic outgroup was *H. boucardi*, *H. dorsalis*, or *H. buccata*, depending on the analysis performed. The third outgroup was *Cyprinella lutrensis*. Allelic codes correspond to those in Table 3. Loci having more than one allele are in parentheses. Abbreviations are as follows: lut = *Cyprinella lutrensis;* buc = *Hybopsis buccata;* dor = *H. dorsalis;* bou = *H. boucardi;* zan = *H. zanema;* hyp = *H. hypsinotus;* rub = *H. rubrifrons;* amb = *H. amblops;* lin = *H. lineapunctata;* amn = *H. amnis;* win = *H. winchelli;* sp. = *H.* sp.

Transformation series	Species and populations of *Hybopsis* and *Cyprinella*											
	lut	buc	dor	bou	zan	hyp	rub	amb	lin	amn	win	sp.
1. M-Acon-A	*b*	*b*	*(bc)*	*b*	*b*	*(ab)*	*(bc)*	*b*	*b*	*b*	*b*	*(ab)*
2. Ada-A	*(bc)*	*(ab)*	*a*	*(abc)*	*a*	*b*	*b*	*(bc)*	*b*	*b*	*(bc)*	*(bc)*
3. Ak-A	*(ab)*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
4. Cbp-1	*b*	*b*	*b*	*a*	*b*	*b*	*b*	*b*	*b*	*c*	*b*	*b*
5. Ck-A	*(bc)*	*(bc)*	*a*	*a*	*a*	*b*	*b*	*b*	*(bc)*	*(cd)*	*(bc)*	*b*
6. Ck-B	*(ac)*	*a*	*a*	*a*	*a*	*a*	*(ab)*	*a*	*a*	*a*	*a*	*a*
7. Ck-C	*b*	*(ce)*	*(ce)*	*a*	*b*	*g*	*(bf)*	*(bd)*	*e*	*e*	*e*	*e*
8. Fum-A	*b*	*c*	*c*	*c*	*a*	*a*	*b*	*b*	*b*	*b*	*b*	*b*
9. G6pdh-B	*b*	*c*	*c*	*a*	*b*	*c*	*c*	*c*	*c*	*c*	*c*	*c*
10. Gpi-A	*(acdf)*	*c*	*(ac)*	*(bf)*	*d*	*b*	*(cdf)*	*(ce)*	*d*	*(ac)*	*(cd)*	*(cdf)*
11. Gpi-B	*(abcfg)*	*c*	*(cf)*	*f*	*(be)*	*f*	*(cf)*	*(defh)*	*f*	*(aef)*	*(fh)*	*(cefh)*
12. Gapdh-A	*(ab)*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*b*	*b*	*b*	*(ab)*
13. G3pdh-A	*(ce)*	*(ac)*	*d*	*c*	*(be)*	*(cf)*	*c*	*c*	*c*	*(bce)*	*c*	*(ce)*
14. M-Icdh-A	*a*	*a*	*a*	*a*	*a*	*a*	*(ab)*	*a*	*a*	*a*	*a*	*a*
15. Ldh-A	*b*	*a*	*a*	*a*	*(ab)*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
16. Ldh-B	*a*	*b*	*a*	*b*	*a*	*b*	*b*	*b*	*b*	*b*	*b*	*b*
17. M-Mdh-A	*(bc)*	*c*	*b*	*a*	*d*	*d*	*d*	*(de)*	*d*	*d*	*d*	*d*

Table 2. Continued.

Transformation series	Species and populations of *Hybopsis* and *Cyprinella*											
	lut	buc	dor	bou	zan	hyp	rub	amb	lin	amn	win	sp.
18. S-Mdh-A	*(ab)*	*(cd)*	*c*	*c*	*e*	*b*	*e*	*e*	*e*	*e*	*e*	*e*
19. S-Mdh-B	*d*	*a*	*a*	*c*	*(abc)*	*a*	*a*	*a*	*a*	*a*	*(ac)*	*a*
20. S-Me-A	*e*	*d*	*d*	*d*	*c*	*d*	*b*	*b*	*b*	*b*	*(ab)*	*b*
21. Pgdh-A	*(de)*	*(de)*	*(adef)*	*g*	*e*	*d*	*(cd)*	*(def)*	*(bd)*	*d*	*d*	*d*
22. Tpi-A	*(bc)*	*(ab)*	*b*	*a*	*b*	*b*	*(ab)*	*(ab)*	*b*	*b*	*b*	*(ab)*
23. Tpi-B[2]	*a*	*a*	*a*	*a*	*a*	*a*	*b*	*b*	*b*	*b*	*b*	*b*
24. Premax process	0	1	1	1	1	1	1	1	1	1	1	1
25. Maxilla process	0	1	1	1	1	1	1	1	1	1	1	1
26. Palatine pocket	0	1	1	1	1	1	1	1	1	1	1	1
27. Maxilla body	0	0	0	1	1	1	1	1	1	1	1	1
28. Mandibular canal	0	0	0	0	1	1	1	1	1	1	1	1
29. Interhyal	0	0	0	0	1	1	1	1	1	1	1	1
30. Hypobranchial 1	0	0	0	0	1	1	1	1	1	1	1	1
31. Basibranchial 1	0	0	0	0	1	1	1	1	1	1	1	1
32. Posterior parasphenoid	0	0	0	0	1	1	1	1	1	1	1	1
33. Orbit shape	0	0	0	0	1	0	1	1	1	1	1	1
34. Urohyal plate	0	0	0	0	0	1	1	1	1	1	1	1
35. Urohyal process	0	0	0	0	0	1	1	1	1	1	1	1
36. Vomerine process	0	0	0	0	0	0	0	1	2	1	1	1
37. Palatine depression	0	0	0	0	0	0	0	0	1	1	1	1

1986). The precept that all available evidence must be brought to bear on any statement about relationships (Kluge, 1989) is also adhered to. Outgroup comparison was used because it is the most comprehensive method for polarizing hypotheses of transformation (Stevens, 1980; Farris, 1982; Kluge, 1984, 1985; Brooks and Wiley, 1985).

Three levels of phylogenetic analysis were performed. The first was a qualitative analysis using PAUP 3.0s (Swofford, 1991). Any transformation series with more than two characters was left unordered. All possible trees were examined using the exhaustive search option. The polymorphic option was used in which PAUP interprets variation observed for a single taxon as having been derived from a monomorphic ancestor. Thus, all but one of the observed characters are considered derived for the terminal taxon. DELTRAN optimization was used because we assumed that there are an essentially infinite number of possible alleles (infinite alleles model) for any locus.

In the second level of analysis, equally parsimonious topologies resulting from each of the outgroup substitutions were analyzed quantitatively using FREQPARS (Swofford and Berlocher, 1987; Swofford, 1988). Both allelic frequencies and morphological transformation series were included; the morphological transformation series were treated as fixed alleles in the taxa in which they occur. Results of this level of analysis are used to discuss the phylogenetic relationships of the ingroup taxa and to examine the evolution of particular transformation series in the third level of analysis.

The third level was a qualitative analysis of character evolution. PAUP forces all polymorphisms to be derived between each terminal taxon and its most recent common ancestor. However, using character argumentation (Wiley et al., 1991) it is possible to reexamine the distribution of these characters and their transformations. For example, when sister taxa share the same polymorphism, it is more parsimonious to assert that the polymorphism was present in their most recent common ancestor. Each polymorphic character was reexamined to determine its most parsimonious distribution on the tree.

RESULTS

Presumptive loci and alleles.—Of the 27 presumptive loci examined, no variation was observed for Cbp-2, Gp-1, Gapdh-B, and Tpi-B[1]. Genotypic frequencies for the 23 variable loci are provided in Table 3.

Qualitative analysis.—Variable alleles and morphological features were combined to produce a qualitative data matrix (Table 2). These data then were analyzed using the outgroup substitution method previously discussed.

PAUP analysis resulted in 12 equally parsimonious 123-step trees when

Table 3. Genotypic distributions for 23 variable loci used in the qualitative phylogenetic analysis of the *Hybopsis amblops* species group.* The first taxonomic outgroup was *H. zanema* and the second taxonomic outgroup was *H. boucardi*, *H. dorsalis*, or *H. buccata*, depending on the analysis performed. Abbreviations are as follows: lut = *Cyprinella lutrensis;* buc = *Hybopsis buccata;* dor = *H. dorsalis;* bou = *H. boucardi;* zan = *H. zanema;* hyp = *H. hypsinotus;* rub = *H. rubrifrons;* amb = *H. amblops;* lin = *H. lineapunctata;* amn = *H. amnis;* win = *H. winchelli;* sp. = *H.* sp.

	Species and populations of *Hybopsis* and *Cyprinella*											
Locus	lut	buc	dor	bou	zan	hyp	rub	amb	lin	amn	win	sp.
M-Acon-A	*bb*(15)**	*bb*(16)	*bb*(14) *bc* (1)	*bb* (7)	*bb*(14)	*ab* (2) *bb* (5)	*bb* (4) *bc* (8) *cc* (3)	*bb*(15)	*bb*(12)	*bb*(13)	*bb*(18)	*ab* (2) *bb*(24)
Ada-A	*bb*(14) *bc* (1)	*aa*(12) *bb* (4)	*aa*(15)	*ab* (1) *bb* (2) *bc* (2) *cc* (2)	*aa*(14)	*bb* (7)	*bb*(15)	*bb*(14) *bc* (1)	*bb*(12)	*bb*(13)	*bb*(16) *bc* (2)	*bb* (1) *bc* (3) *cc*(22)
Ak-A	*aa* (1) *ab* (9) *bb* (5)	*bb*(16)	*bb*(15)	*bb* (7)	*bb*(14)	*bb* (7)	*bb*(15)	*bb*(15)	*bb*(12)	*bb*(13)	*bb*(18)	*bb*(26)
Cbp-1	*bb*(15)	*bb*(16)	*bb*(15)	*aa* (7)	*bb*(14)	*bb* (7)	*bb*(15)	*bb*(15)	*bb*(12)	*cc*(13)	*bb*(18)	*bb*(26)
Ck-A	*bb*(11) *bc* (4)	*bb*(15) *bc* (1)	*aa*(15)	*aa* (7)	*aa*(14)	*bb* (7)	*bb*(15)	*bb*(15)	*bb*(10) *bc* (2)	*cc*(11) *cd* (2)	*bb* (9) *bc* (8) *cc* (1)	*bb*(26)

Table 3. Continued.

	Species and populations of *Hybopsis* and *Cyprinella*											
Locus	lut	buc	dor	bou	zan	hyp	rub	amb	lin	amn	win	sp.
Ck-B	*aa*(14) *ac* (1)	*aa*(16)	*aa*(15)	*aa* (7)	*aa*(14)	*aa* (7)	*aa*(14) *ab* (1)	*aa*(15)	*aa*(12)	*aa*(13)	*aa*(18)	*aa*(26)
Ck-C	*bb*(15)	*ce* (1) *ee*(15)	*ce* (1) *ee*(14)	*aa* (7)	*bb*(14)	*gg* (7)	*bb*(10) *bf* (5)	*bb* (3) *bd* (8) *dd* (4)	*ee*(12)	*ee*(13)	*ee*(18)	*ee*(26)
Fum-A	*bb*(15)	*cc*(16)	*cc*(15)	*cc* (7)	*aa*(14)	*aa* (7)	*bb*(14)	*bb*(15)	*bb*(12)	*bb*(13)	*bb*(18)	*bb*(24)
G6pdh-B	*bb*(15)	*cc*(16)	*cc*(15)	*aa* (7)	*bb*(14)	*cc* (7)	*cc*(15)	*cc*(15)	*cc*(12)	*cc*(13)	*cc*(18)	*cc*(26)
Gpi-A	*ad* (1) *cc* (1) *cd* (6) *cf* (2) *dd* (3) *df* (1) *ff* (1)	*cc*(16)	*ac* (1) *cc*(14)	*bb* (5) *ff* (2)	*dd*(14)	*bb* (7)	*cc* (1) *cd* (8) *cf* (1) *dd* (4) *df* (1)	*cc*(10) *ce* (5)	*dd*(12)	*ac* (2) *cc*(11)	*cc* (1) *cd* (1) *dd*(16)	*cc* (6) *cd* (9) *dd* (9) *df* (1)

Table 3. Continued.

Locus	Species and populations of *Hybopsis* and *Cyprinella*											
	lut	buc	dor	bou	zan	hyp	rub	amb	lin	amn	win	sp.
Gpi-B	*aa* (1) *ab* (1) *ac* (3) *af* (1) *cc* (4) *cf* (3) *cg* (2)	*cc*(16)	*cc* (4) *cf* (8) *ff* (3)	*ff* (7)	*be* (3) *ee*(11)	*ff* (7)	*cf* (2) *ff* (13)	*df* (1) *ef* (1) *ff* (11) *fh* (2)	*ff* (12)	*ae* (1) *ee* (1) *ef* (6) *ff* (5)	*ff* (17) *hh* (1)	*cf* (2) *ef* (1) *ff* (17) *fh* (5) *hh* (1)
Gapdh-A	*aa* (9) *ab* (4) *bb* (2)	*aa*(16)	*aa*(15)	*aa* (7)	*aa*(14)	*aa* (7)	*aa*(15)	*aa*(15)	*bb*(12)	*bb*(13)	*bb*(18)	*ab* (2) *bb*(24)
G3pdh-A	*ce* (1) *ee*(14)	*ac* (4) *cc*(12)	*dd*(15)	*cc* (7)	*be* (5) *ee* (9)	*cc* (6) *ff* (1)	*cc*(15)	*cc*(15)	*cc*(12)	*be* (1) *cc*(12)	*cc*(18)	*cc*(25) *ce* (1)
M-Icdh-A	*aa*(15)	*aa*(16)	*aa*(15)	*aa* (7)	*aa*(14)	*aa* (7)	*aa* (5) *ab* (9) *bb* (1)	*aa*(15)	*aa*(12)	*aa*(13)	*aa*(18)	*aa*(26)
Ldh-A	*bb*(15)	*aa*(16)	*aa*(15)	*aa* (7)	*aa*(13) *ab* (1)	*aa* (7)	*aa*(15)	*aa*(15)	*aa*(12)	*aa*(13)	*aa*(18)	*aa*(26)

Table 3. Continued.

	Species and populations of *Hybopsis* and *Cyprinella*											
Locus	lut	buc	dor	bou	zan	hyp	rub	amb	lin	amn	win	sp.
Ldh-B	*aa*(15)	*bb*(16)	*aa*(15)	*bb* (7)	*aa*(14)	*bb* (7)	*bb*(15)	*bb*(15)	*bb*(12)	*bb*(13)	*bb*(18)	*bb*(26)
M-Mdh-A	*bc* (2) *cc*(13)	*cc*(16)	*bb*(15)	*aa* (7)	*dd*(14)	*dd* (7)	*dd*(15)	*dd*(14) *de* (1)	*dd*(12)	*dd*(13)	*dd*(18)	*dd*(26)
S-Mdh-A	*ab* (7) *bb* (8)	*cc*(10) *cd* (3) *dd* (3)	*cc*(15)	*cc* (7)	*ee*(14)	*bb* (7)	*ee*(15)	*ee*(15)	*ee*(12)	*ee*(13)	*ee*(18)	*ee*(26)
S-Mdh-B	*dd*(15)	*aa*(16)	*aa*(15)	*cc* (7)	*aa* (1) *ab* (4) *ac* (4) *bb* (4) *cc* (1)	*aa* (7)	*aa*(15)	*aa*(15)	*aa*(12)	*aa*(13)	*aa*(16) *ac* (2)	*aa*(26)
S-Me-A	*ee*(15)	*dd*(16)	*dd*(15)	*dd* (7)	*cc*(14)	*dd* (7)	*bb*(15)	*bb*(15)	*bb*(12)	*bb*(13)	*ab* (1) *bb*(17)	*bb*(26)

Table 3. Continued.

	Species and populations of *Hybopsis* and *Cyprinella*											
Locus	lut	buc	dor	bou	zan	hyp	rub	amb	lin	amn	win	sp.
Pgdh-A	*de* (1) *ee*(14)	*dd*(10) *de* (5) *ee* (1)	*aa* (1) *ae* (1) *af* (3) *dd* (2) *de* (1) *df* (2) *ee* (1) *ff* (3)	*gg* (7)	*ee*(14)	*dd* (7)	*cd* (1) *dd*(14)	*dd*(11) *de* (1) *df* (2) *ee* (1)	*bd* (1) *dd*(11)	*dd*(13)	*dd*(18)	*dd*(26)
Tpi-A	*bb* (3) *bc* (8) *cc* (4)	*aa* (4) *ab* (1) *bb*(11)	*bb*(13)	*aa* (7)	*bb*(14)	*bb* (7)	*ab* (2) *bb*(13)	*ab* (1) *bb*(14)	*bb*(12)	*bb*(13)	*bb*(18)	*aa* (1) *ab* (1) *bb*(24)
Tpi-B^2	*aa*(15)	*aa*(16)	*aa*(15)	*aa* (7)	*aa*(14)	*aa* (7)	*bb*(15)	*bb*(15)	*bb*(12)	*bb*(13)	*bb*(18)	*bb*(26)

*Cbp-2, Gp-1, Gapdh-B, and Tpi-B^1 were monomorphic.

**Letters refer to alleles; numbers of individuals scored are in parentheses.

Hybopsis zanema was the first taxonomic outgroup, *H. dorsalis* was the second taxonomic outgroup, and *Cyprinella lutrensis* was the third taxonomic outgroup (Fig. 1). For these trees, the consistency index (CI) (for informative characters) is 0.575; the retention index (RI) is 0.778 and the rescaled consistency index (RC) is 0.727. All topological differences among the trees involve the relationships among *H. lineapunctata, H. amnis, H. winchelli*, and *H.* sp. Eight synapomorphies support the monophyly of the *H. amblops* species group (Fig. 1). These characters are as follows: Ada-A (TS 2-*b*), Ck-A (TS 5-*b*), G6pdh-B (TS 9-*c*), G3pdh-A (TS 13-*c*), Ldh-B (TS 16-*b*), Pgdh-A (TS 21-*d*), a truncated urohyal plate (TS 34-1), and the presence of pointed and thickened urohyal processes (TS 35-1). *Hybopsis hypsinotus* is the sister group of the remaining species of the group. The monophyly of the remaining species is supported by four synapomorphies, all of which are allozymic: Fum-A (TS 8-*b*), S-Mdh-A (TS 18-*e*), S-Me-A (TS 20-*b*), and Tpi-B^2 (TS 23-*b*). Within this clade, *H. rubrifrons* is the most basal lineage. The monophyly of the remaining five taxa is supported by the presence of a posteriorly directed vomerine process (TS 36-1). *Hybopsis amblops* is the sister taxon to the remaining members of this group. The monophyly of *H. lineapunctata, H. amnis, H. winchelli*, and *H.* sp. is supported by three synapomorphies: Ck-C (TS 7-*e*), Gapdh-A (TS 12-*b*), and a deep palatine depression (TS 26-1).

Using *Hybopsis buccata* as the second taxonomic outgroup resulted in 12 equally parsimonious 122-step trees (CI = 0.578; RI = 0.788; RC = 0.743). Six synapomorphies support the *H. amblops* species group in this analysis: G6pdh-B (TS 9-*c*), Gpi-B (TS 11-*f*), Ldh-B (TS 16-*b*), Pgdh-A (TS 21-*d*), and the conditions of the urohyal plate (TS 34-1) and the urohyal process (TS 35-1) (Fig. 2). As in the first analysis using *H. dorsalis* as the second taxonomic outgroup, there was conflict among trees concerning relationships among *H.* sp., *H. amnis, H. winchelli*, and *H. lineapunctata*.

Using *Hybopsis boucardi* as the second taxonomic outgroup resulted in 14 equally parsimonious 124-step trees (CI = 0.586; RI = 0.788; RC = 0.743). Six synapomorphies support the *H. amblops* species group: Ck-A (TS 5-*b*), G6pdh-B (TS 9-*c*), Ldh-B (TS 16-*b*), Pgdh-A (TS 21-*d*), urohyal plate (TS 34-1), and urohyal process (TS 35-1) (Fig. 3). These trees are 14 of the 15 dichotomous resolutions of the clade including *H. lineapunctata, H. amnis, H. winchelli*, and *H.* sp. In all other respects, the relationships among the taxa and the character support for each clade within the *H. amblops* species group are the same as when *H. dorsalis* or *H. buccata* were the second taxonomic outgroups (cf. Figs. 1–3).

In summary, all three analyses are similar in their support for relationships within the *Hybopsis amblops* species group, but differ slightly in their support for the monophyly of the group. Synapomorphies supporting the monophyly of the group that are common to all three analyses include the

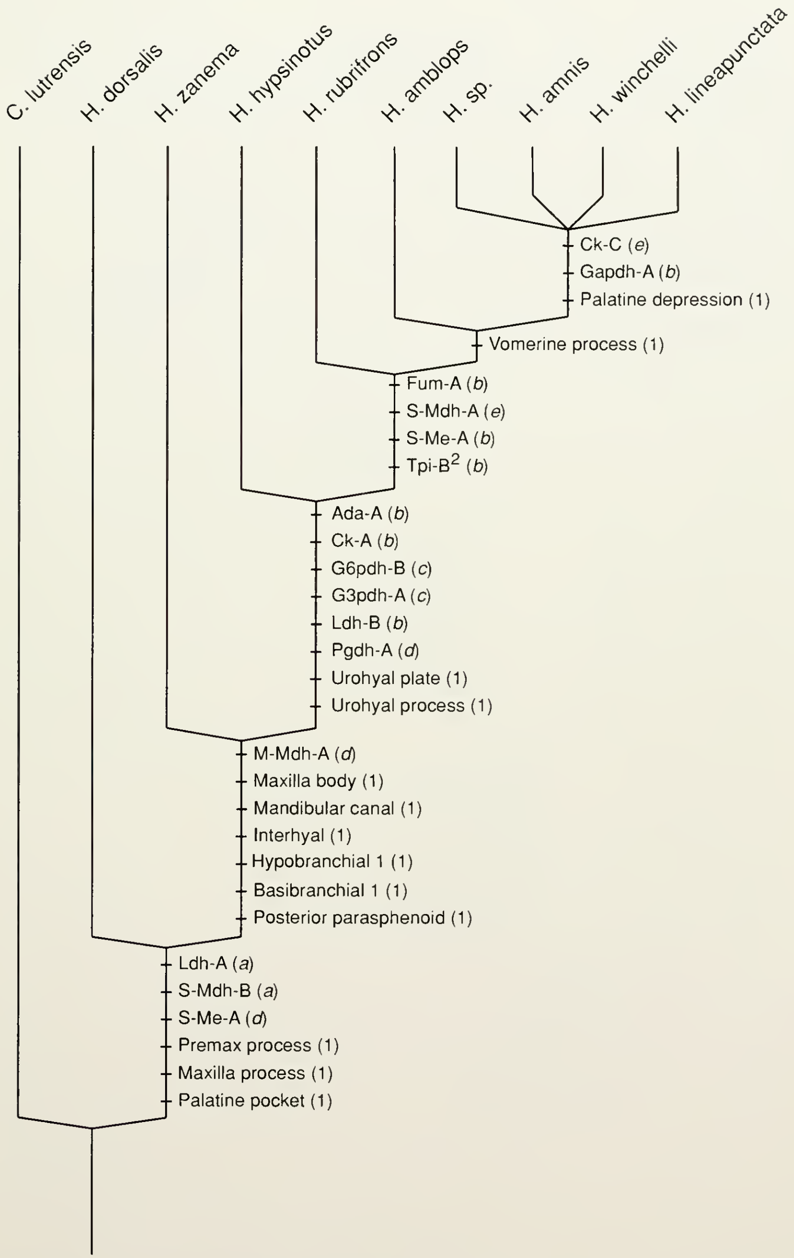

Fig. 1. Strict consensus of 12 equally parsimonious 123-step trees, showing character support for relationships when *Hybopsis dorsalis* is the second taxonomic outgroup. Bars = synapomorphies.

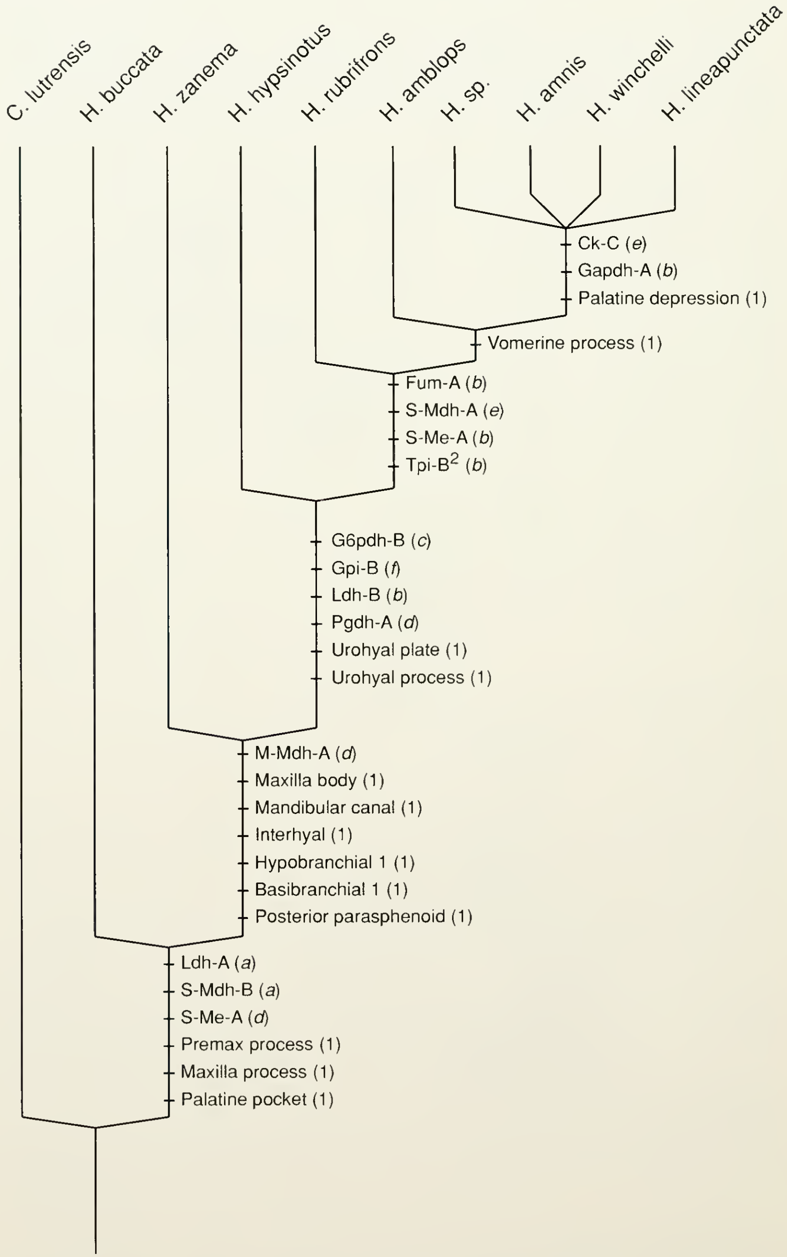

Fig. 2. Strict consensus of 12 equally parsimonious 122-step trees, showing character support for relationships when *Hybopsis buccata* is the second taxonomic outgroup. Bars = synapomorphies.

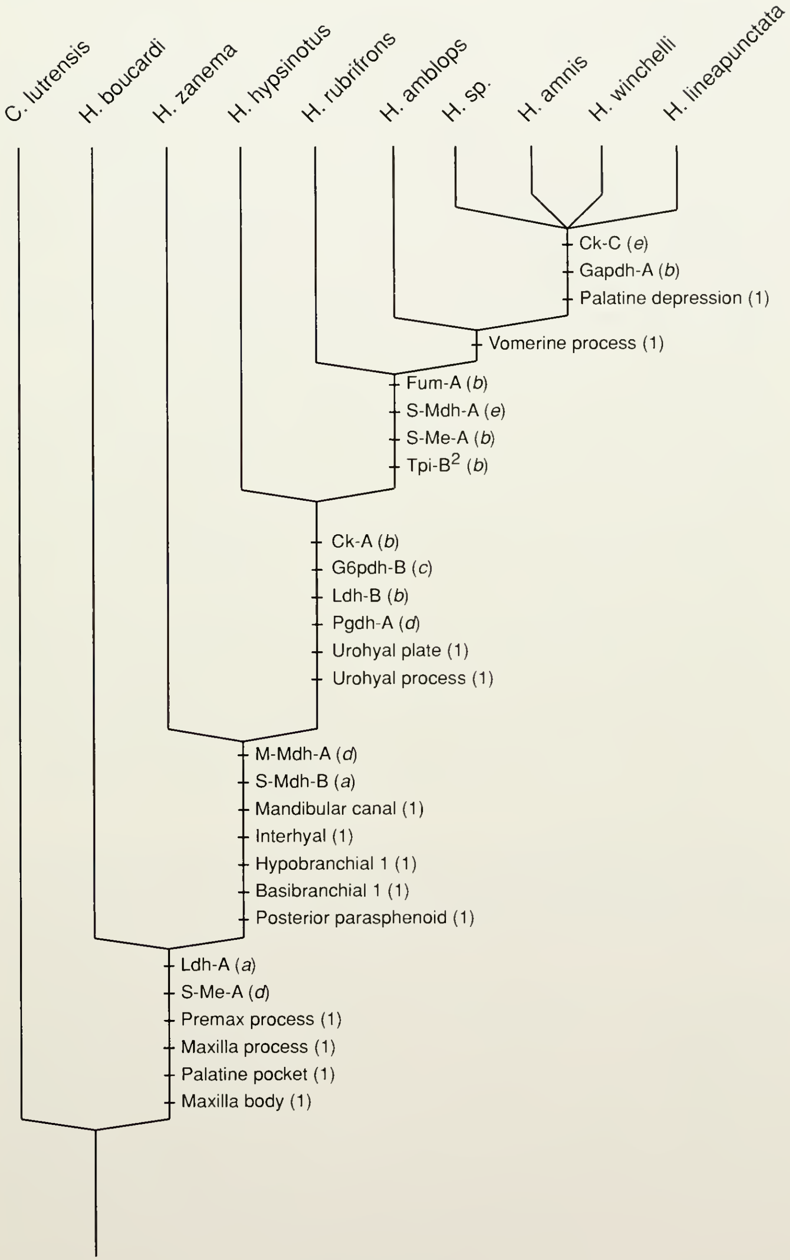

Fig. 3. Strict consensus tree of 14 equally parsimonious 124-step trees, showing character support for relationships when *Hybopsis boucardi* is used as the second taxonomic outgroup. Bars = synapomorphies.

two morphological characters (TS 34-1, TS 35-1) and alleles at three presumptive loci (G6pdh-B, Ldh-B, and Pgdh-A).

Quantitative analysis.—All of the equally parsimonious trees resulting from the qualitative analyses were examined using FREQPARS. In addition, alternate dichotomous resolutions for relationships among *Hybopsis lineapunctata, H. amnis, H. winchelli,* and *H.* sp. were examined. A total of 45 trees was examined, 15 with each substitution of the second outgroup (*H. dorsalis, H. buccata,* or *H. boucardi*). Minor differences in tree lengths were observed, regardless of the outgroup used. Tree lengths varied from 115.72 to 116.78 steps when *H. dorsalis* was the second outgroup, from 114.66 to 115.72 steps when *H. buccata* was the second outgroup, and from 122.90 to 123.96 steps when *H. boucardi* was the second outgroup.

Regardless of which taxon (*Hybopsis dorsalis, H. buccata,* or *H. boucardi*) was used as the second outgroup, the topology of the shortest tree is the same (Fig. 4). However, in each case, only 0.26 steps separates this tree from the next shortest tree, and there was a difference of only slightly more than one step across all 15 trees. These small differences in tree lengths indicate that the resolution of relationships among *H. lineapunctata, H. amnis, H. winchelli,* and *H.* sp. are tenuous.

Character analysis.—Exclusive of the synapomorphies discussed for the qualitative analyses, there are seven instances in which a derived allele completely replaces the plesiomorphic one. This evolutionary replacement results in fixed autapomorphies. *Hybopsis lineapunctata* is autapomorphic in its possession of Gpi-A (TS 10-*d*). Cbp-1 (TS 4-*c*) is uniquely fixed in *H. amnis. Hybopsis hypsinotus* appears to be autapomorphic for five alleles: Ck-C (TS 7-*g*), Fum-A (TS 8-*a*), Gpi-A (TS 10-*b*), S-Mdh-A (TS 18-*b*), and S-Me-A (TS 20-*d*).

Character argumentation in conjunction with DELTRAN optimization provides evidence that some polymorphisms have persisted through speciation events. Other resolutions of the *Hybopsis* sp. + *H. amnis* + *H. winchelli* + *H. lineapunctata* clade result in a maximum of seven polymorphisms postulated to have persisted through speciation events. Two of these character-state optimizations, however, conflict with one another. Ada-A (TS 2-*bc*) is shared by *H. amblops, H. winchelli,* and *H.* sp.; Ck-A (TS 5-*bc*) is shared by *H. lineapunctata* and *H. winchelli;* and Gpi-A (TS 10-*cd*) is shared by *H. rubrifrons, H. winchelli,* and *H.* sp. Either Gpi-B (TS 11-*fh*) is shared by *H. amblops, H. winchelli,* and *H.* sp. or Gpi-B (TS 11-*ef*) is shared by *H. amblops, H.* sp., and *H. amnis.* G3pdh-A (TS 13-*ce*) is shared by *H. amnis* and *H.* sp. *Hybopsis rubrifrons, H. amblops,* and *H.* sp. share Tpi-A (TS 22-*ab*). Three polymorphisms persisting through speciation events are evident using the shortest tree from the quantitative analysis: Ck-A (TS 5-*bc*), Gpi-A (TS 10-*cd*) and Gpi-B (TS 11-*ef*). Polymorphisms at Ada-A, G3pdh-A, and Tpi-A would be considered terminal autapomorphies (Fig. 5).

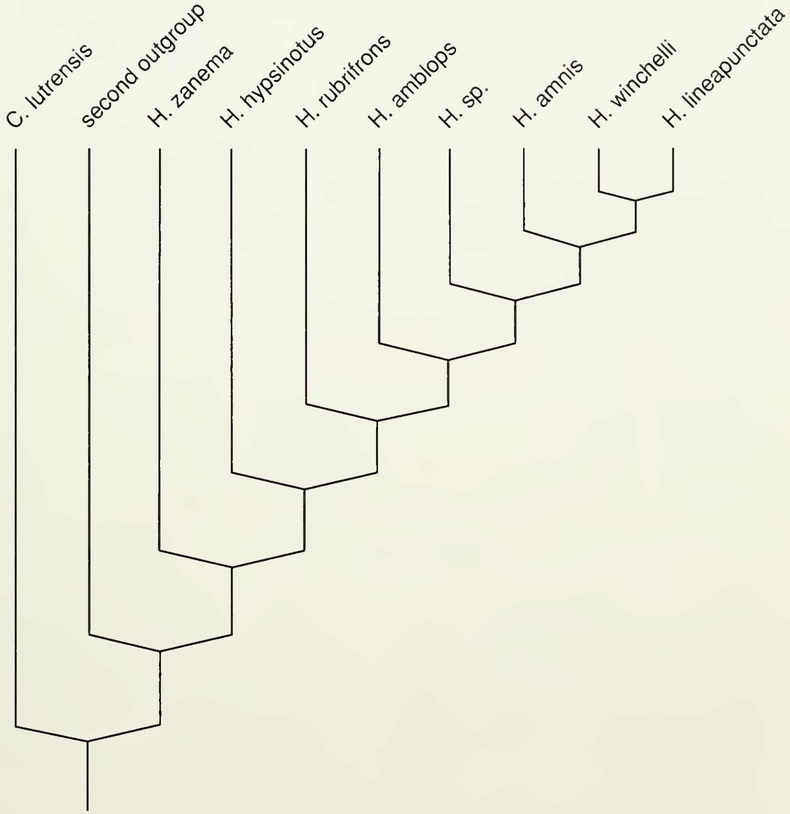

Fig. 4. Shortest tree topology resulting from FREQPARS analyses, regardless of the second outgroup. Tree length is 115.72 steps when *Hybopsis dorsalis* is the second outgroup, 114.66 steps if *H. buccata* is the second outgroup, and 122.90 steps if *H. boucardi* is the second outgroup.

After character analysis, some polymorphic characters continue to exhibit at least one autapomorphic allele in terminal taxa. For example, *Hybopsis hypsinotus* has both *a* and *b* alleles for the M-Acon-A locus (TS 1), but it is autapomorphic for only the *a* allele. In all, there are 36 such terminal autapomorphic polymorphisms in the *H. amblops* species group when DELTRAN optimization and character argumentation were used. In contrast, character analysis indicated only four derived losses of alleles.

DISCUSSION

This analysis resolves the relationships of *Hybopsis rubrifrons* and *H. hypsinotus*, which, previously, were considered part of a trichotomy with

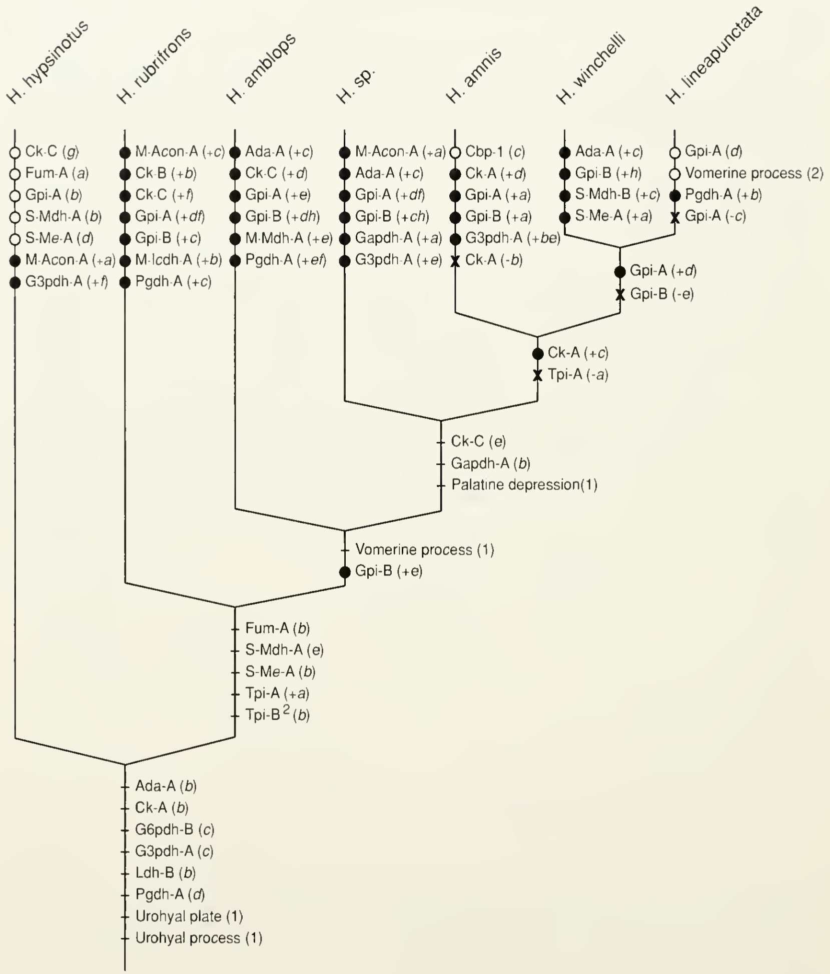

Fig. 5. Tree showing the character distributions for the ingroup taxa, after character argumentation and DELTRAN optimization of polymorphisms. Bars = synapomorphies; open circles = fixed autapomorphies; closed circles = autapomorphic and persistent (synapomorphic) polymorphisms; crosses = loss of polymorphic alleles.

the remainder of the *H. amblops* species group (Mayden, 1989). However, the relationships among *H.* sp., *H. amnis*, *H. winchelli*, and *H. lineapunctata* were not well resolved. Although the shortest tree resulting from the FREQPARS analysis postulates that *H. winchelli* is the sister to *H. lineapunctata*, that these two together are the sister to *H. amnis*, and that these three are the sister to *H.* sp., this tree is only 0.26 steps shorter than the next shortest tree and 1.06 steps shorter than the longest possible resolution of this group. Most of the allelic variation among these taxa is distributed along terminal branches with few character changes along internodes. Short internodes are consistent with an evolutionary pattern of very short time intervals between speciation events. Additional character sets will be necessary to further evaluate species relationships and character evolution in this clade.

Phylogenetic relationships among members of the *Hybopsis amblops* species group are relevant to the biogeographic history of the Gulf Coastal region. Wiley and Mayden (1985) found that many pairs of aquatic vertebrates along the northern Gulf Coast are sister species, with one species occurring in the Mobile Bay Basin and drainages to the west and the other species occurring in drainages to the east of the Mobile Bay Basin. Although the distributions of *H. winchelli* and *H.* sp. are consistent with this vicariant pattern, our analyses suggest that they are not sister taxa and their common ancestor could not have participated in this vicariance event. The distributions and sister group relationship of *H. amnis* and *H. lineapunctata* + *H. winchelli* are consistent with a Mobile Bay vicariance event. Our analysis also suggests a possible vicariance event above and below the Fall Line within the Mobile River system. *Hybopsis lineapunctata* is found above the Fall Line in the Coosa and Tallapoosa rivers, whereas its sister, *H. winchelli*, is found below the Fall Line in the Mobile River system and in Gulf Drainages as far east as Florida. Wiley and Mayden (1985) suggested that *H. amnis* and *H. amblops* might be part of a group of species whose vicariance event is loosely associated with the Mississippi River. However, we found no support for a sister group relationship between these species, suggesting that these species were not affected by this vicariance event.

Clemmer (1980) stated that *Hybopsis* sp. might be a species distinct from *H. winchelli* based on differences in the snout shape, mouth position, head length, and size and position of nuptial tubercles. Clemmer (1971; 1980) also suggested that *H.* sp. was most closely related to *H. winchelli*. Allelic variation provides little additional information concerning the taxonomic status of *H.* sp. All other terminal taxa in this study have at least one unique character (although this character is not always fixed), whereas *H.* sp. does not exhibit any unique characters. We found no fixed allozymic differences between *H. winchelli* and *H.* sp., but there were allelic frequency differences at 10 loci. Although the relationship of *H.* sp. to *H. amnis*, *H. winchelli*, and *H. lineapunctata* is tenuous, the shortest tree does not support a sister-group relationship between *H.* sp. and *H. winchelli*. Given these relationships, *H.* sp. should be described as a new species because it most closely resembles *H. winchelli*, which is not its sister taxon, and seems to be an independent lineage (Fig. 5). Autapomorphies for *H.* sp. would be more readily inferred if this species is found to be the sister to the clade including *H. amnis*, *H. winchelli*, and *H. lineapunctata*.

Outgroup substitution for the second outgroup resulted in identical ingroup topologies but character support for the monophyly of the *Hybopsis amblops* species group differed. When *H. buccata* was the second outgroup, character states Ada-A (TS 2-*b*), Ck-A (TS 5-*b*), and G3pdh-A (TS 13-*c*) did not support the monophyly of the *H. amblops group*. When *H. dorsalis* was the second outgroup, character state Gpi-B (TS 11-*f*) was not a synapomorphy for this clade. Likewise, when *H. boucardi* was the second outgroup, Ada-A (TS 2-*b*), Gpi-B (TS 11-*f*), and G3pdh-A (TS 13-*c*) were not synapomorphies.

The allozymic and morphological data provide complementary evidence for relationships in the *Hybopsis amblops* species group. Support for the clade including *H. rubrifrons*, *H. amblops*, *H.* sp., *H. amnis*, *H. winchelli*, and *H. lineapunctata* is entirely based on allozymic characters, as are the relationships among *H. amnis*, *H. winchelli*, and *H. lineapunctata*. However, morphological characters showed less homoplasy (cf. Fig. 5 and Table 2) and stabilized stems whenever they appeared as synapomorphies. Some stems are supported by similar numbers of morphological and allozymic characters, such as the clade including *H.* sp., *H. amnis*, *H. winchelli*, and *H. lineapunctata*, indicating areas of congruence between these character sets. Polymorphisms also lend support to clades when they persist through speciation events, such as electromorph Gpi-B (TS 11-*ef*), which supports the clade comprised of *H. amblops*, *H.* sp., *H. amnis*, *H. winchelli*, and *H. lineapunctata*.

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LITERATURE CITED

Agassiz, J. L. R. 1854. Notice of a collection of fishes from the southern bend of the Tennessee River, in the State of Alabama. Am. J. Sci. Arts, 17:297–308, 353–365.

Bailey, R. M. 1951. A check list of the fishes of Iowa with keys for identification. Pp. 187–238 *in* Harlow, J. R., and E. B. Speaker (eds), *Iowa Fish and Fishing*. Des Moines: Iowa State Conservation Commission.

Bailey, R. M., J. E. Fitch, E. S. Herald, E. A. Lachner, C. C. Lindsey, C. R. Robins, and W. B. Scott. 1970. *A List of Common and Scientific Names of Fishes from the United States and Canada*. 3rd ed. Am. Fish. Soc. Spec. Pub. 6:1–150.

Brooks, D. R., and E. O. Wiley. 1985. Theories and methods in different approaches to phylogenetic systematics. Cladistics 1:1–12.

Burr, B. M., and R. M. Mayden. 1992. Phylogenetics and North American Fishes. Pp. 18–75 *in* Mayden, R. L. (ed), *Systematics, Historical Ecology, & North American Freshwater Fishes.* Stanford: Stanford Univ. Press.

Buth, D. G. 1983. Duplicate isozyme loci in fishes: origins, distribution, phyletic consequences and locus nomenclature. Pp. 381–400 *in* Rattazzi, M. C., J. G. Scandalios, and G. S. Whitt (eds), *Isozymes: Current Topics in Biological and Medical Research.* Vol. 10. New York: Alan R. Liss.

Churchill, S. P., E. O. Wiley, and L. A. Hauser. 1985. Biological realities and the proper methodology: a reply to Duncan. Taxon 34:124–130.

Clemmer, G. H. 1971. The systematics and biology of the *Hybopsis amblops* complex. Doctoral dissertation. New Orleans: Tulane Univ.

Clemmer, G. H. 1980. *Notropis winchelli* (Hay). P. 195 *in* Lee, D. S., et al. (eds), *Atlas of North American Freshwater Fishes.* Raleigh: North Carolina State Mus. Nat. Hist.

Clemmer, G. H., and R. D. Suttkus. 1971. *Hybopsis lineapunctata,* a new cyprinid fish from the Upper Alabama River system. Tulane Stud. Zool. Bot. 17(2): 21–30.

Coburn, M. M. 1982. Anatomy and relationships of *Notropis atherinioides.* Doctoral dissertation. Columbus: Ohio State Univ.

Coburn, M. M. and T. M. Cavender. 1992. Interrelationships of North American Cyprinid Fishes. Pp. 328–373 *in* R. L. Mayden (ed), *Systematics, Historical Ecology, & North American Freshwater Fishes.* Stanford: Stanford Univ. Press.

Cope, E. D. 1870. A partial synopsis of the fishes of the fresh waters of North Carolina. Proc. Am. Philos. Soc. 11:448–495.

Dimmick, W. W. 1993. A molecular perspective on the phylogenetic relationships of the barbeled minnows, historically assigned to the genus *Hybopsis* (Cyprinidae: Cypriniformes). Mol. Phylo. Evol. 2(3):173–184.

Donoghue, M. J., and P. D. Cantino. 1984. The logic and limitations of the outgroup substitution approach to cladistic analysis. Syst. Bot. 9(2):192–202.

Farris, J. S. 1982. Outgroups and parsimony. Syst. Zool. 31:328–334.

Farris, J. S. 1983. The logical basis of phylogenetic systematics. Pp. 7–36 *in* Platnick, N. I., and V. A. Funk (eds), *Advances in Cladistics.* Vol. 2. New York: Columbia Univ. Press.

Farris, J. S., and A. G. Kluge. 1985. Parsimony, synapomorphy, and explanatory power: a reply to Duncan. Taxon 34:130–135.

Farris, J. S., and A. G. Kluge. 1986. Synapomorphy, parsimony, and evidence. Taxon 35:298–306.

Fildes, R. A., and H. Harris. 1966. Genetically determined variation of adenylate kinase in man. Nature (London) 209:261–263.

Girard, C. 1857. Researches upon the cyprinoid fishes inhabiting the fresh waters of the United States, west of the Mississippi Valley, from specimens in the museum of the Smithsonian Institute. Proc. Acad. Nat. Sci. Philadelphia (1856) 8(5):165–213.

Hennig, W. 1966. *Phylogenetic Systematics.* Chicago: Univ. Illinois Press.

Hubbs, C. L. 1951. *Notropis amnis,* a new cyprinid fish of the Mississippi fauna, with two subspecies. Univ. Michigan Mus. Zool. Occas. Pap. 530:1–31.

International Union of Biochemistry Nomenclature Committee. 1984. Enzyme Nomenclature. Orlando: Academic Press.

Jenkins, R. E., and E. A. Lachner. 1971. Criteria for analysis and interpretation of the American fish genera *Nocomis* Girard and *Hybopsis* Agassiz. Smithsonian Contrib. Zool. 90:1–15.

Jordan, D. S. 1877. A partial synopsis of the fishes of the upper Georgia, with supplementary papers on the fishes of Tennessee, Kentucky, and Indiana. Ann. New York Lynceum Nat. Hist. 11:307–377.

Jordan, D. S. 1924. Concerning the genus *Hybopsis* of Agassiz. Copeia 1924 (130):51–52.

Jordan, D. S. 1929. *Manual of the Vertebrate Animals of the Northeastern United States, inclusive of marine species.* 13th ed. Yonkers-on-Hudson: World Book Co.

Jordan, D. S. and B. W. Evermann. 1896. The fishes of North and Middle America: A descriptive catalogue of the species of fish-like vertebrates found in the waters of North America, north of the Isthmus of Panama. Part 1. Bull. US Natl. Mus. 47:1–954.

Jordan, , D. S., B. W. Evermann, and H. W. Clark. 1930. *Check list of the fishes and fish-like vertebrates of North and Middle America, north of the northern boundary of Venezuela and Colombia.* Rept. US Bur. Fish. 1055:1–670.

Kluge, A. G. 1984. The relevance of parsimony to phylogenetic inference. Pp. 24–38 *in* Duncan, T., and T. Stuessy (eds), *Cladistics: Perspectives on the Reconstruction of Evolutionary History.* New York: Columbia Univ. Press.

Kluge, A. G. 1985. Ontogeny and phylogenetic systematics. Cladistics 1:13–28.

Kluge, A. G. 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Syst. Zool. 38:7–25.

Kluge, A. G., and J. S. Farris. 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. 18:1–32.

Lachner, E. A. and R. E. Jenkins. 1967. Systematics, distribution, and evolution of the chub genus *Nocomis* (Cyprinidae) in the southwestern Ohio River Basin, with the description of a new species. Copeia 1967(3): 557–580.

Lachner, E. A. and R. E. Jenkins. 1971. Systematics, distribution, and evolution of the chub genus *Nocomis* Girard (Pisces, Cyprinidae) of eastern United States, with descriptions of new species. Smithsonian Contrib. Zool. 85:1–97.

Lee, D. S., C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer, Jr. 1980 et seq. *Atlas of North American Freshwater Fishes.* Raleigh: North Carolina State Mus. Nat. Hist.

Mayden, R. L. 1989. Phylogenetic studies of North American minnows, with emphasis on the genus *Cyprinella* (Teleostei: Cypriniformes). Univ. Kansas Mus. Nat. Hist. Misc. Publ. 80:1–189.

Mayden, R. L., B. M. Burr, L. M. Page, and R. R. Miller. 1992. The native freshwater fishes of North America. Pp. 827–863 *in* Mayden, R. L., (ed), *Systematics, Historical Ecology, & North American Freshwater Fishes.* Stanford: Stanford Univ. Press.

Murphy, R. W., J. W. Sites, Jr., D. G. Buth, and C. H. Haufler. 1990. Proteins I: isozyme electrophoresis. Pp. 45–126 *in* Hillis, D. M., and C. Moritz (eds), *Molecular Systematics.* Sunderland: Sinauer Associates.

Nelson, J. S. 1994. *Fishes of the World*. 3rd ed. New York: Wiley Interscience.

Page, L. M. and Burr, B. M. 1991. *A Field Guide to Freshwater Fishes: North America north of Mexico*. Boston: Houghton Mifflin Co.

Rafinesque, C. S. 1820. *Ichthyologicia Ohiensis, or Natural History of the Fishes Inhabiting the River and Its Tributary Streams, Preceded by a Physical Description of the Ohio and Its Branches*. Lexington: W. G. Hunt.

Reno, H. W. 1969. A partial nomenclatural review of *Hybopsis* (Pisces: Cyprinidae) Proc. Oklahoma Acad. Sci. Biol. Sci. 48:65–71.

Robins, C. R., R. M. Bailey, C. E. Bond, J. R. Brooker, E. A. Lachner, R. N. Lea, and W. B. Scott. 1980. *A List of Common and Scientific Names of Fishes from the United States and Canada*. 4th ed. Am. Fish. Soc. Spec. Publ. 12:1–174.

Robins, C. R., R. M. Bailey, C. E. Bond, J. R. Brooker, E. A. Lachner, R. N. Lea, and W. B. Scott. 1991. *Common and Scientific Names of Fishes from the United States and Canada*. 5th ed. Am. Fish. Soc. Spec. Publ. 20:1–183.

Selander, R. K., M. H. Smith, S. Y. Yang, W. E. Johnson, and J. R. Gentry. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Stud. Genet. IV. Univ Texas Publ. 7103:49–90.

Stevens, P. F. 1980. Evolutionary polarity of character states. Ann. Rev. Ecol. Syst. 11:333–358.

Swofford, D. L. 1988. FREQPARS: Frequency Analysis Using Parsimony. Version 1.0. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Swofford, D. L. 1991. PAUP: Phylogenetic Analysis Using Parsimony, Version 3.0s. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.

Swofford, D. L., and S. H. Berlocher. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Syst. Zool. 36:293–325.

Wiley, E. O. 1981. *Phylogenetics: The Theory and Practice of Phylogenetic Systematics*. New York: John Wiley and Sons.

Wiley, E. O., and R. L. Mayden. 1985. Species and speciation in phylogenetic systematics, with examples from the North American fish fauna. Ann. Missouri Bot. Gard. 72:596–635.

Wiley, E. O., and T. A. Titus. 1992. Phylogenetic relationships among members of the *Hybopsis dorsalis* species group (Teleostei: Cyprinidae). Univ. Kansas Mus. Nat. Hist. Occas. Pap. 152:1–18.

Wiley, E. O., D. Siegel-Causey, D. R. Brooks, and V. A. Funk. 1991. *The Compleat Cladist: A Primer of Phylogenetic Procedures*. Univ. Kansas Mus. Nat. Hist. Spec. Publ. 19:1–158.

APPENDIX—SPECIMENS EXAMINED

All material examined is deposited in the ichthyological collection of the Natural History Museum of The University of Kansas. Vouchers for the electrophoretic analysis include the remains of actual specimens (number of specimens indicated in parentheses).

Cyprinella lutrensis.—Osage River Drainage: KU 22985 (9), Kansas: Franklin Co.: Marais des Cygnes River, 2.8 mi E Ottawa, S Hwy 68. Missouri River Drainage: KU 22986 (6), Kansas: Douglas Co.: Kansas River at Lawrence, just E Bowersock Dam.

Hybopsis buccata.—Pascagoula River Drainage: KU 22987 (2), Mississippi: Perry Co.: Black Creek at Moody's Landing, 6.0 mi E Brooklyn. Escambia River Drainage: KU 22988 (6), Florida: Escambia Co.: Pine Barren Creek at Hwy 29, just S Pine Barren. Yellow River Drainage: KU 22989 (8), Alabama: Covington Co.: Yellow River at Hwy 84, E Andalusia.

Hybopsis dorsalis.—Missouri River Drainage: KU 23008 (15), Kansas: Atchison Co.: Deer Creek at Hwy 7, ca. 1.5 mi N Atchison.

Hybopsis boucardi.—Balsas River Drainage: KU 22990 (7), MEXICO: Oaxaca: Río Mixteco, 24.4 mi S Chazumba (1.6 mi S Cuyotepyi).

Hybopsis zanema.—Broad River Drainage: KU 22991 (14), South Carolina: Newberry Co.: Indian Creek, at junction Hwys 176 and 121, 8 mi S Whitmire.

Hybopsis hypsinotus.—Rocky River Drainage: KU 22992 (1), North Carolina: Mecklenburg Co.: Mallard Creek on Mallard Creek Road. KU 22993 (6), North Carolina: Mecklenburg Co.: Mallard Creek.

Hybopsis rubrifrons.—Savannah River Drainage: KU 22994 (15), Georgia: Banks Co.: Grove Creek at Hwy 51, 2.7 mi SE Lula. ex UAIC 7959.

Hybopsis amblops.—Tennessee River Drainage: KU 22995 (15), Tennessee: Lewis Co.: Buffalo River at mouth Grinder's Creek. ex UAIC 7966.

Hybopsis lineapunctata.—Alabama River Drainage: KU 22996 (12), Alabama: Bibb Co.: Cahaba River at Co. Rd. 27. ex UAIC 7884.

Hybopsis amnis.—Red River Drainage: KU 22145 (13 of 14), Louisiana: Ouachita Parish: Ouachita River at Lazarres Park, W Monroe.

Hybopsis winchelli.—Black Warrior River Drainage: KU 22997 (9), Alabama: Tuscaloosa Co.: Mill Creek at Hwy 82, just W Tuscaloosa. ex UAIC 7914. KU 22998 (6), Alabama: Tuscaloosa Co.: Mill Creek at Hwy 82, just W Tuscaloosa. Cahaba River Drainage: KU 22999 (3), Alabama: Perry Co.: Waters Creek, ca. 15 mi NW Selma on Hwy 14.

Hybopsis sp.—Yellow River Drainage: KU 21895 (1 of 2), Florida: Okaloosa Co.: Big Horse Creek at Hwy 2, 2.5 mi E junction with Hwy 189. KU 23002 (5), Alabama: Covington Co.: Yellow River at Hwy 84, E Andalusia. KU 23006 (1), Florida: Okaloosa Co.: Big Horse Creek at County Road 2, ca. 3 mi E Blackman. Perdido River Drainage: KU 23000 (4), Florida: Escambia Co.: Brushy Creek just W Walnut Hill, 1.5 mi W junction of Hwys 97A and 99A on Hwy 99A. KU 23001 (4), Alabama: Baldwin Co.: Hollinger Creek at junction with dirt road 3 mi W Hwy 112. Escambia River Drainage: KU 23003 (1), Florida: Escambia Co.: Big Escambia Creek at Century, at Fannie Road on W side. Conecuh River Drainage: KU 23004 (5), Alabama: Butler Co.: Hawkins Creek at County Road 37, 5 mi S Greenville. Choctawhatchee River Drainage: KU 23005 (5), Alabama: Geneva Co.: Flat Creek at Hwy 153, 4.5 mi S and 4 mi W Samson.

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